Cell and Tissue Research (2021) 385:115–126
https://doi.org/10.1007/s00441-021-03441-8

REGULAR ARTICLE

LncRNA KCNQ1OT1 attenuates osteoarthritic chondrocyte dysfunction via the miR-218-5p/PIK3C2A axis

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Received: 19 August 2020 / Accepted: 1 March 2021 / Published online: 30 March 2021

Abstract

The occurrence of osteoarthritis is closely related to chondrocyte dysfunction caused by cellular inflammatory response and matrix degradation, which seriously affect the quality of life of patients. Therefore, this study aimed to investigate the role of potassium voltage-gated channel subfamily Q member 1 overlapping transcript 1 (KCNQ1OT1), a member of the lncRNA voltage-gated channel subfamily Q, in the development of osteoarthritis. In this study, RT-qPCR results showed that KCNQ1OT1 expression was downregulated in osteoarthritic chondrocytes compared with normal chondrocytes. In addition, upregulation of KCNQ1OT1 significantly enhanced the viability of osteoarthritic chondrocytes, inhibited cell apoptosis, and reduced the release of inflammatory cytokines and metal matrix enzymes. Next, bioinformatics analysis and luciferase reporter gene analysis predicted and validated the targeting relationship between KCNQ1OT1 and miR-218-5p. We found that the expression of miR-218-5p was significantly upregulated in osteoarthritic chondrocytes, and knockdown of miR-218-5p significantly enhanced the viability of osteoarthritic chondrocytes, inhibited apoptosis, and decreased the abundance of inflammatory cytokines and metal matrix enzymes. Furthermore, the targeting relationship between miR-218-5p and recombinant phosphoinositide-3-kinase class-2-alpha polypeptide (PIK3C2A) was identified, and overexpression of PIK3C2A enhanced cell viability, and reduced apoptosis and secretion of inflammatory factors. Finally, we found that miR-218-5p overexpression reversed the protective effect of overexpression of KCNQ1OT1 or PIK3C2A on osteoarthritic chondrocytes. In conclusion, our results demonstrated that KCNQ1OT1 upregulated PIK3C2A and activated the PI3K/AKT/mTOR pathway to reduce chondrocyte dysfunction by targeting miR-218-5p, providing new insights into the pathogenesis of osteoarthritis.

Keywords Osteoarthritis · LncRNA KCNQ1OT1 · MiR-218-5p · PIK3C2A · The PI3K/AKT/ mTOR pathway

Abbreviations

LncRNA	Long non-coding RNA
siRNA	Small interference RNA
ceRNA	Competing endogenous RNA
KCNQ1OT1	Potassium voltage-gated channel subfamily Q member 1 overlapping transcript 1
PIK3C2A	Recombinant phosphoinositide-3-kinase class-2-alpha polypeptide
IL-1β	Interleukin-1β
IL-6	Interleukin-6
TNF-α	Tumor necrosis factor-α
COX-2	Cyclooxygenase-2
MMP-13	Matrix metallopeptidase-13
ADAMTS-5	ADAM metallopeptidase with thrombospondin type 1 motif 5
COL2A1	Collagen type II alpha 1 chain
ECM	Extracellular matrix components
OARSI	Osteoarthritis Research Society International
SDS-PAGE	Sodium dodecyl sulfate polyacrylamide gel electrophoresis
PVDF	Polyvinylidene difluoride
HRP	Horseradish peroxidase

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Introduction

Osteoarthritis is a common orthopedic disease, which can cause such symptoms as chronic progressive joint swelling, pain, joint stiffness, and even deformity (Liu et al. 2018). Among the middle-aged people aging 50–60, the prevalence of osteoarthritis has reached 50% and 80% among the elderly over 75 (Safiri et al. 2020; Sun et al. 2019). Osteoarthritis has a progressive development with the increase of age, and the late disability rate can be as high as 40%, which seriously affects the health and quality of life of the elderly (Zhang et al. 2016). Unfortunately, the pathogenesis of the disease is not completely clear, and the results of clinical treatment are not ideal. Although the implementation of artificial joint replacement has alleviated the development of osteoarthritis to some extent, however, there are currently no ideal measurements to treat osteoarthritis. Therefore, it is of great significance for the effective and reasonable prevention and treatment of osteoarthritis to clarify the pathogenesis and progress mechanism of osteoarthritis and to find key targets for intervention.

Cytokines play an important role in physiological metabolism and functional regulation of organisms. In the early stage of osteoarthritis, inflammatory factors already exist. Among various inflammatory factors, interleukin-1β (IL-1β) and tumor necrosis factor-α (TNF-α) are most closely related to osteoarthritis (Yao et al. 2020). At present, there is a large amount of evidence that the contents of IL-1β and TNF-α in knee joint fluid are positively related to the severity of osteoarthritis. Chen et al. found that the expression of IL-1β and TNF-α was significantly increased in severe osteoarthritis compared with that in normal bone joints (Chen et al. 2015). Cartilage erosion is a sign of osteoarthritis, which is caused by the increased expression of extracellular matrix degrading enzymes and the decreased expression of extracellular matrix components. Matrix metalloproteinase-13 (MMP-13) and Adam metalloproteinase with thrombospondin type 1 motif 5 (ADAMTS-5) are two of the most important extracellular matrix degrading enzymes (Mehana et al. 2019; Wang et al. 2017). Collagen type II alpha 1 chain (COL2A1) is the main component of cartilage extracellular matrix (Yassin et al. 2020). The gene expression of extracellular matrix degrading enzymes and extracellular matrix components is regulated by a variety of extracellular metabolic factors including IL-1β, IL-6 and TNF-α.

LncRNAs are a sub-type of non-coding RNAs with a length of more than 200 nt and exhibit higher tissue specificity and cell specificity than coding RNAs. They have different expression in different cells, tissues, and developmental stages and are involved in the occurrence and development of various diseases. More and more studies showed that lncRNAs are involved in regulating the pathogenesis of osteoarthritis. Microarray analysis had found that over 500 lncRNAs are abnormally expressed in osteoarthritis cartilage tissues (Fu et al. 2015; Xing et al. 2014). It has been reported that lncRNAs CHRF and CXCR4 can participate in regulating inflammatory response signaling pathways (Li et al. 2018; Yu et al. 2019). In addition, lncRNAs HOTAIR and MALAT1 can degrade cartilage extracellular matrix components (ECM) by increasing the expression of matrix metalloproteinases (MMPs), leading to cartilage degeneration and osteoarthritis (Zhang et al. 2016; Zhang et al. 2019).

Potassium voltage-gated channel subfamily Q member 1 overlapping transcript 1 (KCNQ1OT1) is a candidate gene for type II diabetes, which is believed to play a role in impaired insulin function (Chen et al. 2018). Previous a study has found that KCNQ1OT1 can promote the expression of β-Catenin and promote the proliferation and migration of ovarian cancer cells (Sunamura et al. 2016). Besides, it is reported that KCNQ1OT1 contributes to the progression and chemoresistance in acute myeloid leukemia by modulating Tspan3 through suppressing miR-193a-3p (Sun et al. 2020). Recently, a study showed that resveratrol can upregulate KCNQ1OT1 through the Wnt/β-Catenin pathway, reduce mice osteolysis induced by polymethylmethacrylate, and promote osteogenic differentiation of mouse mesenchymal stem cells in vitro, suggesting that KCNQ1OT1 may play an important role in inflammatory bone diseases (Gao et al. 2018).

In this study, we clarified the expression of KCNQ1OT1 in osteoarthritic chondrocytes and explored its possible mechanism in the progression of osteoarthritis, aiming to find a potential target for the treatment of osteoarthritis.

Materials and methods

Samples

Osteoarthritic cartilage tissue samples included in this study were obtained from patients undergoing knee replacement surgery ($n = 25$, 65.2 ± 4.5 years). Non-osteoarthritic cartilage tissue samples were obtained from trauma patients ($n = 25$, 62.7 ± 3.6 years). The protocol of this study was approved by the ethics committee of the first hospital of Jilin University, and written informed consent was obtained from each participant.

Chondrocyte culture and transfection

Chondrocytes were isolated from cartilage tissue of osteoarthritic patients undergoing arthroplasty and trauma patients

without osteoarthritis. Articular cartilage was digested consecutively in DMEM with pronase and collagenase as described previously until the cartilaginous tissue mass had largely disappeared and the solution had become cloudy, and then the medium was filtered with 75-μM cell strainer to remove impurities. Next, the supernatant was discarded after centrifugation at 1000 r/min for 5 min, and DMEM medium (containing 10% fetal bovine serum, penicillin 100 U/mL, streptomycin 100 U/mL) was added, and the cell density was adjusted to 1×10^6 mL and seeded in culture flasks, which were further incubated at 37 °C in a 5% CO_2 saturated humidity incubator (Lu et al. 2017). When the cells grew to about 80% confluency, they were passaged at a ratio of 1:2 after digestion with 0.25% trypsin.

The adenoviral KCNQ1OT1 vector (Ad-KCNQ1OT1) and PIK3C2A overexpression vector (pcDNA-PIK3C2A) and their negative controls were purchased from RiboBio Co., Ltd (Guangzhou, China). The short interfering RNAs (siRNAs) against KCNQ1OT1 (si-KCNQ1OT1) and PIK3C2A (si-PIK3C2A) and their negative controls were synthesized by Bioneer (Shanghai, China). The miR-218-5p mimic, miR-218-5p inhibitor, and their negative controls were purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Transfection was performed using Lipofectamine 2000 transfection reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions.

RNA extraction and real-time quantitative PCR analysis

Total RNA was isolated from osteoarthritic chondrocytes by using the TRIzol (Invitrogen, Carlsbad, CA, USA). Single-stranded cDNA was synthesized with the PrimeScript Reagent Kit (Promega, USA). Real-time qPCR was conducted by using a SYBR Premix Ex TaqTM Kit (Takara, Dalian, China). The reaction was run in ABI7500 Real-time PCR system (Applied Biosystems, Carlsbad, CA). GAPDH and U6 were used as endogenous controls. Briefly, 2 μL of cDNA was added to 10 μL of the 2 × SYBR green PCR master mix with 0.4 μL of Taq polymerase enzyme (RiboBio, China), 0.8 μL of each primer, and 6 μL ddH_2O to a final volume of 20 μL. The qPCR cycling conditions consisted of: 95 °C for 2 min, then 35 cycle amplification for 20 s at 95 °C, 30 s at 55 °C, and 15 s at 72 °C, followed by 1 min at 72 °C. The primers used in this study were synthesized by Sangon Biotech (Shanghai, China). The primer sequences were as follows: KCNQ1OT1 forward, 5′-ACT CAC TCA CTC ACT CAC T-3′, reverse, 5′-CTG GCT CCT TCT ATC ACA TT-3′; miR-218-5p forward, 5′-TTG CGG ATG GTT CCG TCA AGC A-3′, reverse, 5′-ATC CAG TGC AGG GTC CGA GG-3′; PIK3C2A forward, 5′-CTT ACT CAT TGC TTC ACC AGT GG-3′, reverse, 5′-GCC TCA ATC CAG GTC ACA GCT A-3′; U6 forward, 5′-CTC GCT TCG GCA GCA CA-3′, reverse, 5′-AAC GCT TCA CGA ATT TGC GT-3′; GAPDH forward, 5′-ATC ACT GCC ACC CAG AAG AC-3′; reverse: 5′-TTT CTA GAC GGC AGG TCA GG-3′. The relative expression levels were calculated by using the $2^{-\Delta\Delta CT}$ method.

Luciferase reporter gene assay

StarBase (http://starbase.sysu.edu.cn/) was used to predict potential target genes of KCNQ1OT1, and miR-218-5p is screened as the research object. In addition, PIK3C2A is one target gene of miR-218-5p. The wild type and mutant sequences of KCNQ1OT1 or PIK3C2A were cloned into the PGL3 luciferase reporter vector (Promega, USA), respectively. HEK293 cells were seeded in 24-well plates, when growing to approximately 70% confluence, and co-transfected with luciferase plasmids and miR-218-5p mimic or NC-mimic by using Lipofectamine 2000. After 48-h transfection, the luciferase activity was determined by Multiskan FC Microplate Reader (Thermo Fisher Scientific, Waltham, MA, USA).

MTT assay

Cell viability was determined by MTT kits (Dojindo, Kumamoto, Japan). The osteoarthritic chondrocytes were seeded in a 96-well plate at 1.5×10^4 cells/well. On days 1, 2, 3, and 4 after transfection, respectively, the supernatant was discarded and 20 μL of MTT solution was added to each well. After incubating at 37 °C for 4 h, the absorbance at 450 nm was measured and recorded. Each experimental procedure was processed at least three times.

Flow cytometry

The cells were collected and washed twice with PBS. The cell suspension (100 μL) with a cell density of 1×10^6 cells/mL was transferred into the culture tube, then incubated with 5 μL Annexin V-FITC and 5 μL propidium iodide with room temperature for 20 min in the dark. Finally, 400 μL of binding buffer was added, and apoptotic cells were determined by flow cytometry (BD Biosciences, USA).

ELISA

The secretion of IL-6, TNF-α, and COX-2 in osteoarthritic chondrocytes supernatant was detected by enzyme-linked immunosorbent assay (ELISA) kits (Sigma, St. Louis, MO, USA) following the manufacturer's instructions. Similarly, the levels of secretory proteins including MMP-13, ADAMTS-5, and COL2A1 were detected by ELISA kits (Sigma, St. Louis, MO, USA).

Western blotting

Protein homogenates from osteoarthritic chondrocytes were extracted as previously described. Briefly, the cells were lysed for 20 min on ice in ice-cold lysis buffer (Roche). The lysates were centrifuged at 12,000 × *g* for 20 min at 4 °C to obtain a clear lysate. The protein content of each sample was determined by using the BCA Protein Assay Kit (Thermo Scientific). Then, equal amounts of proteins (15 μg/lane) were separated on a 12% sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to polyvinylidenedifluoride (PVDF) membranes (Bio-Rad, Hercules, CA, USA). The membranes were blocked in 5% (w/v) nonfat dry milk in TBST (Tris-buffered saline-0.1%Tween) at 25 °C for 3 h and then incubated with the following primary antibodies: rabbit polyclonal anti-β-actin antibody (1:900, Abcam), rabbit polyclonal anti-PIK3C2A antibody (1:600, Abcam), rabbit polyclonal anti-AKT antibody (1:1500, Abcam), rabbit polyclonal anti-p-AKT antibody (1:500, Abcam), rabbit monoclonal anti-PI3K antibody (1:1000, Abcam), and rabbit monoclonal anti-p-PI3K antibody (1:600, Abcam). Then, the membranes were incubated

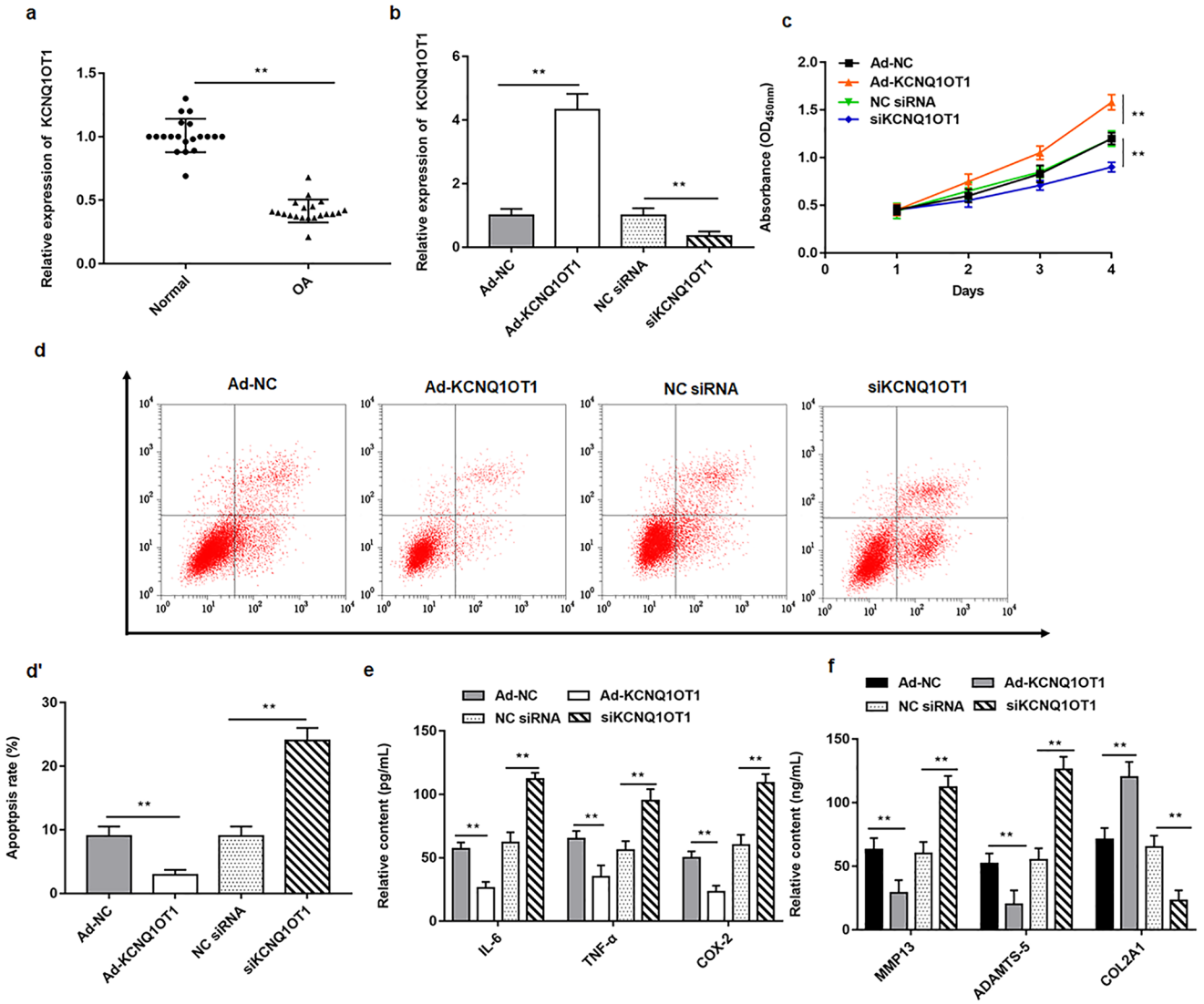

Fig. 1 The expression of KCNQ1OT1 was downregulated in osteoarthritic chondrocytes. **a** The chondrocytes derived from osteoarthritic cartilage tissue and non-osteoarthritic cartilage tissue were collected ($N=50$, 25 normal and 25 osteoarthritis). Relative expression of KCNQ1OT1 was detected by RT-qPCR. **b** Ad-KCNQ1OT1 and siKCNQ1OT1 were transfected into osteoarthritic chondrocytes, respectively. And relative expression of KCNQ1OT1 was detected by RT-qPCR. **c** The viability of osteoarthritic chondrocytes was analyzed by MTT assay. **d** and **d′** Apoptosis of osteoarthritic chondrocytes was detected by flow cytometry. **e** Relative content of IL-6, TNF-α, and COX-2 were analyzed by ELISA kits. **f** ELISA was used to detect the secretion levels of MMP-13, ADAMTS-5, and COL2A1. $N=3$, $^{**}P<0.01$

with horseradish peroxidase (HRP)-conjugated goat anti-rabbit IgG (1:3000, Abcam, ab6721) or goat anti-mouse IgG (1:3000, Abcam, ab6728) for 1 h at room temperature. The bands were visualized by using an ECL Plus Chemiluminescence Reagent Kit (Pierce, Rockford, IL, USA) and were photographed by a chemiluminescence imaging system. Image J software was used to quantify the band densities.

Statistical analysis

All statistical analyses were performed by using the SPSS software (ver. 21.0; SPSS, Chicago, IL). The quantitative data derived from three independent experiments were expressed as mean ± SEM. Comparisons between two groups were made by the Student's *t*-test. Data between multiple groups were performed with one-way analysis of variance (ANOVA) followed by post hoc analysis with LSD test. $P < 0.05$ was considered statistically significant.

Results

The expression of KCNQ1OT1 was downregulated in osteoarthritic chondrocytes

We detected KCNQ1OT1 expression in chondrocytes derived from osteoarthritic cartilage tissue and non-osteoarthritic cartilage tissue, respectively, and the results suggested that KCNQ1OT1 expression in osteoarthritic chondrocytes was downregulated (Fig. 1a). For further reveal the role of KCNQ1OT1 in osteoarthritic chondrocytes, the KCNQ1OT1 expression vector and KCNQ1OT1 siRNA were transfected into osteoarthritic chondrocytes. RT-qPCR results showed that Ad-KCNQ1OT1 significantly upregulated KCNQ1OT1 expression, while KCNQ1OT1 siRNA inhibited KCNQ1OT1 expression (Fig. 1b). Next, furthermore, we observed that KCNQ1OT1 promoted osteoarthritic chondrocyte viability (Fig. 1 c), inhibited apoptosis (Fig. 1d, d′) and inflammatory factors secretion including IL-6, TNF-α and COX-2 (Fig. 1e). Conversely, interfering with KCNQ1OT1 expression inhibited cell viability and promoted apoptosis and inflammatory factor secretion. Besides, we found that KCNQ1OT1 inhibited MMP-13 and ADAMTS-5 secretion and promoted COL2A1 secretion, whereas interference with KCNQ1OT1 promoted MMP-13 and ADAMTS-5 secretion and inhibited COL2A1 secretion (Fig. 1f).

MiR-218-5p was a direct target of KCNQ1OT1

It has been reported that miR-218-5p can promote the osteogenic differentiation of bone marrow mesenchymal stem cells to improve the osteoporosis of menopausal mice, suggesting a potential role of miR-218-5p in bone diseases (Kou et al. 2020). Therefore, we investigated the possible relationship between KCNQ1OT1 and miR-218-5p. We found a highly conserved binding sequence between miR-218-5p and KCNQ1OT1 through online bioinformatics database retrieval (Fig. 2a). In addition, the luciferase reporter gene

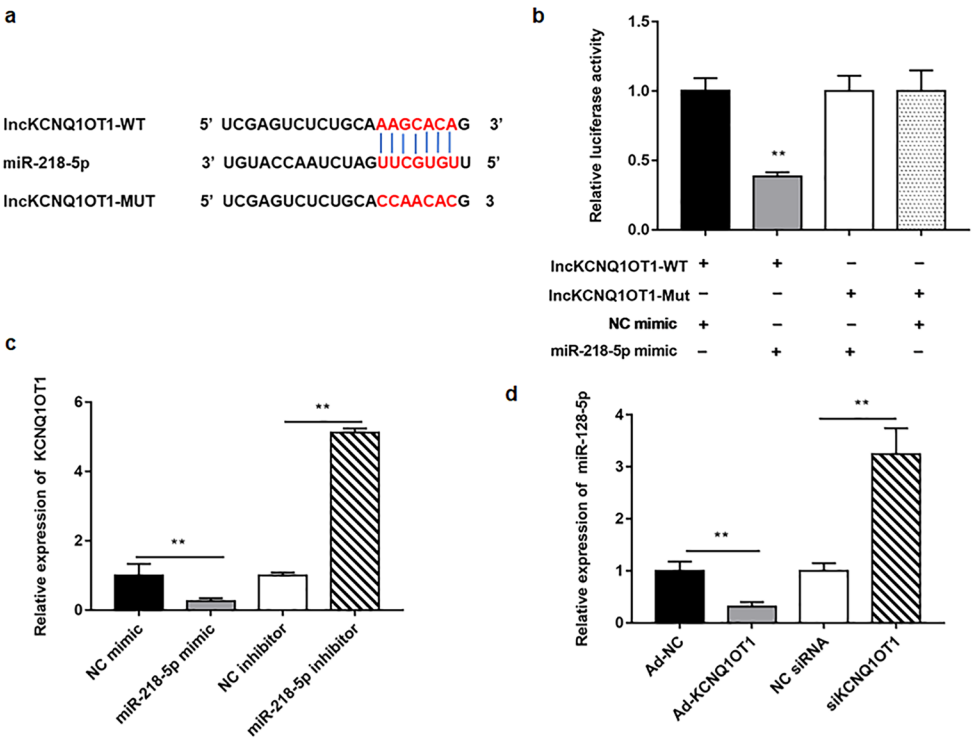

Fig. 2 MiR-218-5p was a direct target of KCNQ1OT1. **a** StarBase 3.0 was used to predicts the targeting site of KCNQ1OT1 and miR-218-5p. **b** Relative luciferase activity was tested with wild-type and mutant-type KCNQ1OT1, respectively. **c** MiR-218-5p mimic and miR-218-5p inhibitor were transfected into osteoarthritic chondrocytes, respectively, and relative expression of KCNQ1OT1 was detected by RT-qPCR. **d** Relative expression of miR-218-5p was detected by RT-PCR after KCNQ1OT1 overexpressed or knocked down. $N = 3$, $^{**}P < 0.01$

assay results showed that miR-218-5p mimic significantly reduced the luciferase activity of KCNQ1OT1-wt reporter, but had no significant effect on the luciferase activity of KCNQ1OT1-mut reporter (Fig. 2b). To further verify the targeting relationship between KCNQ1OT1 and miR-218-5p, we transfected miR-218-5p mimic and miR-218-5p inhibitor into osteoarthritic chondrocytes, and RT-qPCR results showed that miR-218-5p mimic significantly downregulated the expression of KCNQ1OT1 (Fig. 2c). Next, the Ad-KCNQ1OT1 and KCNQ1OT1 siRNA were transfected into osteoarthritic chondrocytes, and RT-qPCR results showed that the expression of miR-218-5p was increased compared with the control group (Fig. 2d).

The expression of miR-218-5p was upregulated in osteoarthritic chondrocytes

Our previous findings indicated that the expression of KCNQ1OT1 in osteoarthritic chondrocytes was downregulated, so we supposed that the expression of miR-218-5p in osteoarthritic chondrocytes should be upregulated. We detected miR-218-5p expression in chondrocytes derived from osteoarthritic cartilage tissue and non-osteoarthritic cartilage tissue, respectively, and the results suggested that miR-218-5p expression in osteoarthritic chondrocytes was upregulated (Fig. 3a). The miR-218-5p mimic and miR-218-5p inhibitor were transfected into osteoarthritic chondrocytes, and

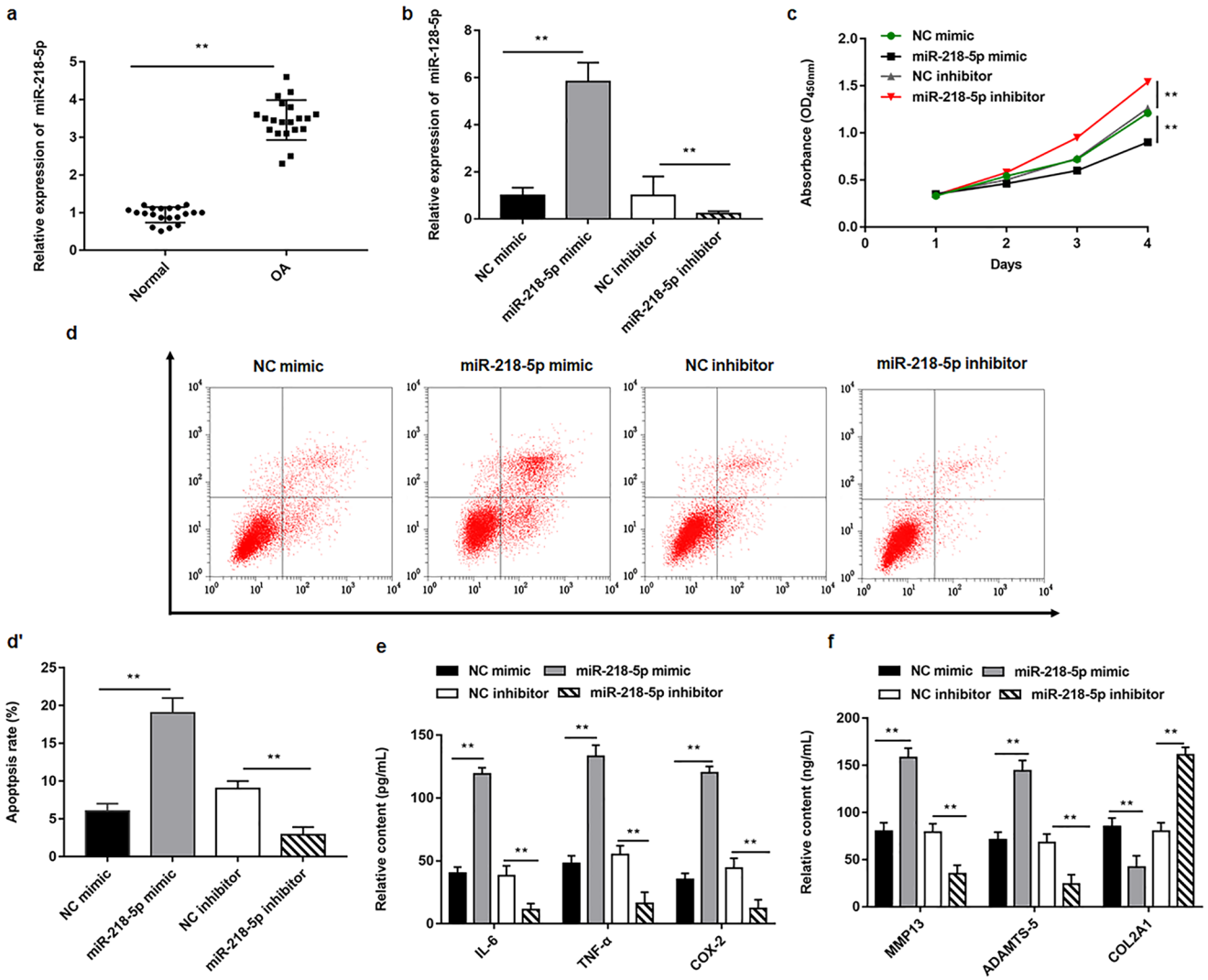

Fig. 3 The expression of miR-218-5p was upregulated in osteoarthritic chondrocytes. **a** Relative expression of miR-218-5p in normal chondrocytes and osteoarthritic chondrocytes was detected by RT-qPCR. **b** MiR-218-5p mimic and miR-218-5p inhibitor were transfected into osteoarthritic chondrocytes, respectively. **c** Osteoarthritic chondrocyte viability was analyzed by MTT assay. **d** and **d′** Apoptosis of osteoarthritic chondrocytes was detected by flow cytometry. **e** Relative content of IL-6, TNF-α, and COX-2 were analyzed by ELISA kits. **f** ELISA was used to detect the secretion levels of MMP-13, ADAMTS-5, and COL2A1. $N=3$, $^{**}P<0.01$

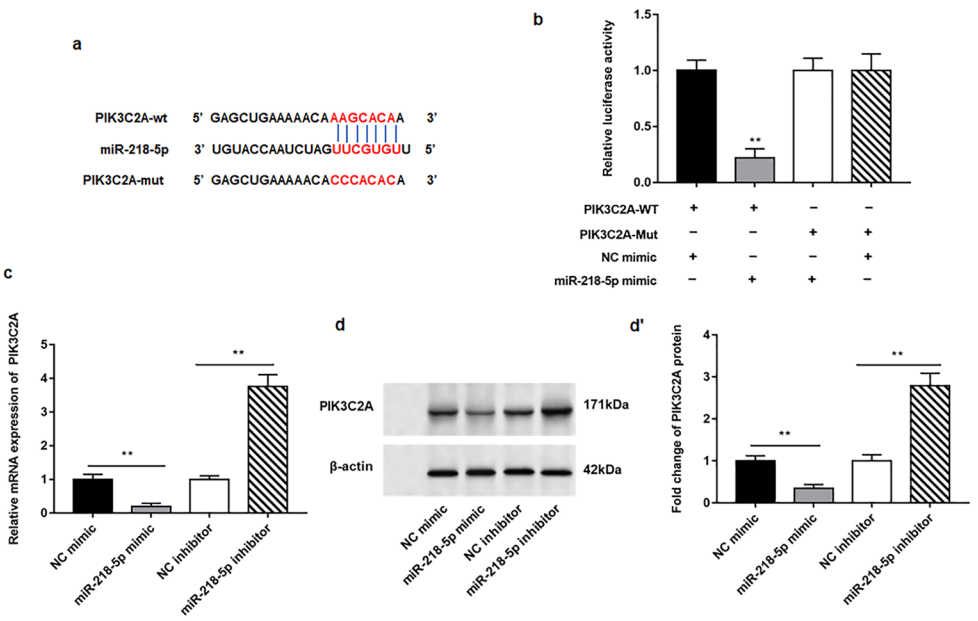

Fig. 4 MiR-218-5p directly targeting 3′UTR of PIK3C2A. **a** StarBase 3.0 was used to predicts the targeting site of miR-218-5p in PIK3C2A mRNA 3′UTR. **b** The relative luciferase activity was tested with wild-type and mutant-type PIK3C2A, respectively. **c, d** and **d′** The miR-218-5p mimic or miR-218-5p inhibitor were transfected into osteoarthritic chondrocytes and the mRNA and protein expression of PIK3C2A were analyzed by RT-qPCR and Western botting. β-actin was used as an invariant internal control for calculating protein-fold changes. $N=3$, $^{**}P<0.01$

transfection efficiency is shown in Fig. 3b. Furthermore, we observed that miR-218-5p mimic inhibited osteoarthritic chondrocyte viability (Fig. 3c) and promoted apoptosis (Fig. 3d, d′) and inflammatory factors secretion including IL-6, TNF-α, and COX-2 (Fig. 3e). Conversely, miR-218-5p inhibitor promoted cell viability and inhibited apoptosis and inflammatory factor secretion. As expected, miR-218-5p mimic promoted MMP-13 and ADAMTS-5 secretion and inhibited COL2A1 secretion, whereas miR-218-5p inhibitor reduced MMP-13 and ADAMTS-5 secretion and increased COL2A1 secretion (Fig. 3f).

MiR-218-5p regulated proliferation and apoptosis of osteoarthritic chondrocytes by targeting PIK3C2A

It has been reported that PIK3C2A as a potential target of miR-218-5p can reduce the secretion of matrix degrading enzymes including MMP13 and ADAMTS-5 and promote the production of collagen II to reduce the dysfunction of chondrocytes, which suggests that PIK3C2A may be a potential target for the treatment of osteoarthritis (Lu et al. 2017). To further explore the regulatory relationship between miR-218-5p and PIK3C2A, firstly, we predicted possible target genes for miR-218-5p through Starbase 3.0 and found that miR-218-5p directly targets the 3′UTR of PIK3C2A. The wild-type and the mutant-type sequences of PIK3C2A were shown in Fig. 4a. Next, luciferase reporter gene analysis verified the targeting relationship between miR-218-5p and PIK3C2A (Fig. 4b). In addition, RT-qPCR and Western blotting results also showed that miR-218-5p mimic significantly reduced the mRNA (Fig. 4c) and protein (Fig. 4d, d′) levels of PIK3C2A.

Next, the pcDNA-PIK3C2A and PIK3C2A siRNA were transfected into osteoarthritic chondrocytes, respectively. Western blotting results showed that PIK3C2A siRNA significantly reduced the protein expression of PIK3C2A (Fig. 5a, a′). MTT assay results showed that PIK3C2A siRNA significantly inhibited chondrocyte viability (Fig. 5b). As expected, transfection of si-PIK3C2A significantly promoted apoptosis (Fig. 5c, c′). In addition, ELISA results suggested that PIK3C2A siRNA significantly promoted the secretion of inflammatory factors including IL-6, TNF-α, and COX-2 (Fig. 5d). Besides, several important secretory proteins in osteoarthritic chondrocytes were examined with ELISA kits and the results showed that PIK3C2A siRNA promoted the secretion of MMP-13 and ADAMTS-5, while the expression of COL2A1 was significantly inhibited (Fig. 5e).

MiR-218-5p functioned through the PI3K/AKT/mTOR pathway by targeting PIK3C2A

To explore whether miR-218-5p functioned through the PI3K/AKT/mTOR pathway by targeting PIK3C2A, the osteoarthritic chondrocytes were transfected with miR-218-5p mimic alone or together with pcDNA-PIK3C2A. RT-qPCR results showed that pcDNA-PIK3C2A reversed the inhibition effect of miR-218-5p on PIK3C2A expression (Fig. 6a). MTT assay results showed that pcDNA-PIK3C2A reversed the inhibition effect of miR-218-5p on chondrocyte viability (Fig. 6b). In addition, pcDNA-PIK3C2A significantly reduced the secretion of IL-6, TNF-α, and COX-2 (Fig. 6c). Next, ELISA results showed that pcDNA-PIK3C2A significantly reversed the increased effect of miR-218-5p mimic on MMP-13 and ADAMTS-5 and the

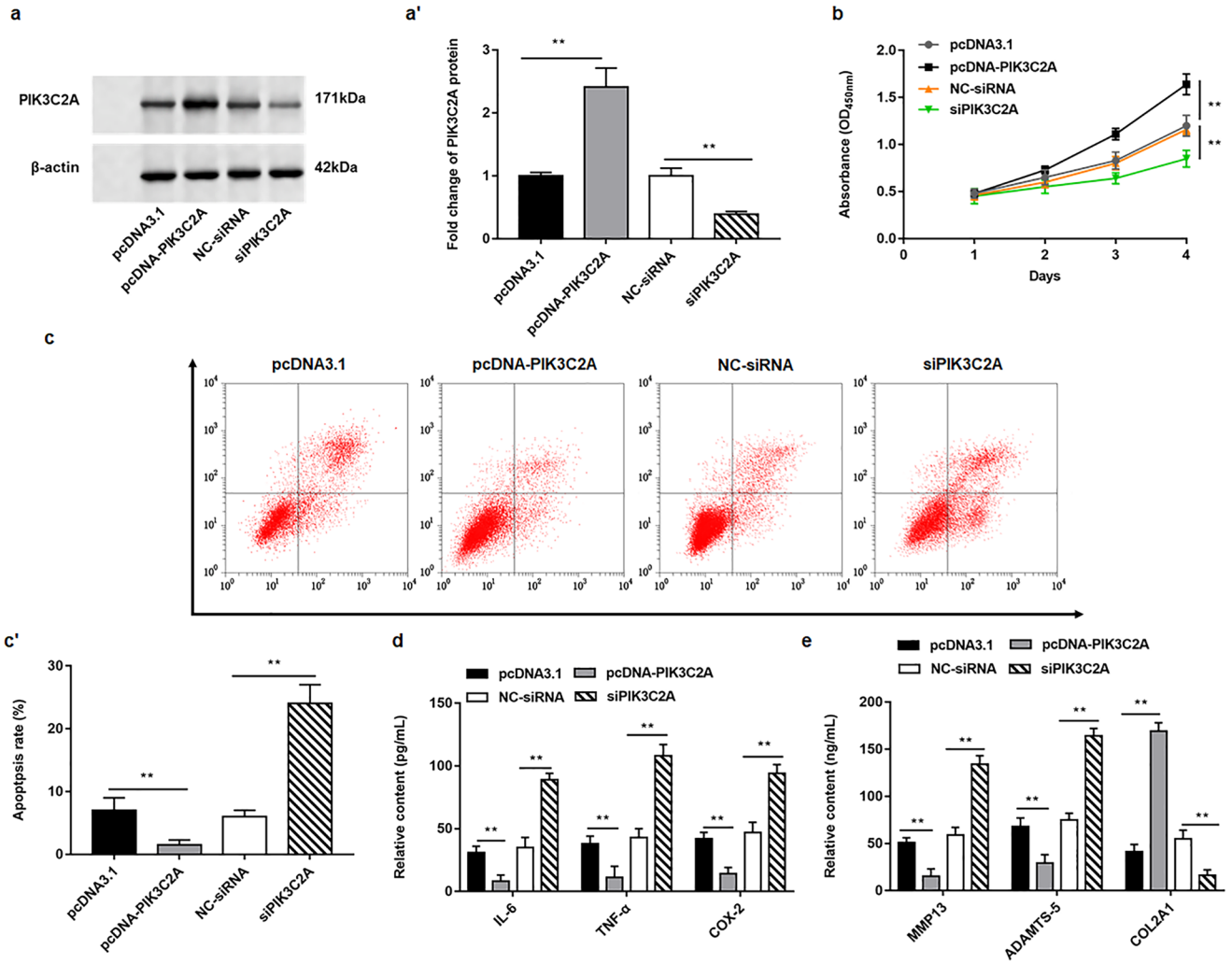

Fig. 5 The regulatory effect of PIK3C2A on osteoarthritic chondrocytes. Osteoarthritic chondrocytes were transfected with pcDNA3.1, pcDNA-PIK3C2A, NC-siRNA, and PIK3C2A siRNA, respectively. **a** and **a′** Relative protein expression of PIK3C2A was analyzed by Western botting. **b** MTT assay was used to measure the viability of osteoarthritic chondrocytes. **c** and **c′** Osteoarthritic chondrocyte apoptosis was detected by flow cytometry. **d** Relative content of IL-6, TNF-α, and COX-2 were analyzed by ELISA kits, respectively. **e** ELISA was used to detect the secretion levels of MMP-13, ADAMTS-5, and COL2A1. β-actin was used as the loading control. $N=3$, $^{**}P<0.01$

decreased effect of COL2A1 (Fig. 6d). Besides, we detected the phosphorylation levels of PI3K, Akt, and mTOR, and Western blotting results showed that the phosphorylation levels of PI3K, Akt, and mTOR were decreased when miR-218-5p mimic was overexpress, whereas pcDNA-PIK3C2A significantly reversed the decreased effect of miR-218-5p mimic on the phosphorylation levels of PI3K, Akt and mTOR (Fig. 6e, e′).

KCNQ1OT1 attenuated chondrocyte dysfunction by activating the PI3K/Akt/mTOR pathway by targeting miR-218-5p

The PI3K/Akt/mTOR signaling pathway is closely related to inflammation and plays an important regulatory role in the osteoarthritis progression. To further investigate whether KCNQ1OT1 was involved in osteoarthritis pathogenesis through the regulation of the PI3K/AKT/mTOR pathway, osteoarthritic chondrocytes were transfected with Ad-KCNQ1OT1 alone or together with miR-218-5p mimic. Meanwhile, the expression of miR-218-5p as well as the PI3K/Akt/mTOR pathway-related proteins was analyzed. RT-qPCR results showed that miR-218-5p mimic reversed the inhibition effect of KCNQ1OT1 on miR-218-5p expression (Fig. 7a). The results of MTT assay showed that miR-218-5p mimic significantly reversed the promotion effect of KCNQ1OT1 on chondrocyte viability (Fig. 7b). In addition, pcDNA-PIK3C2A significantly increased the secretion of IL-6, TNF-α, and COX-2 (Fig. 7c). Similarly, miR-218-5p

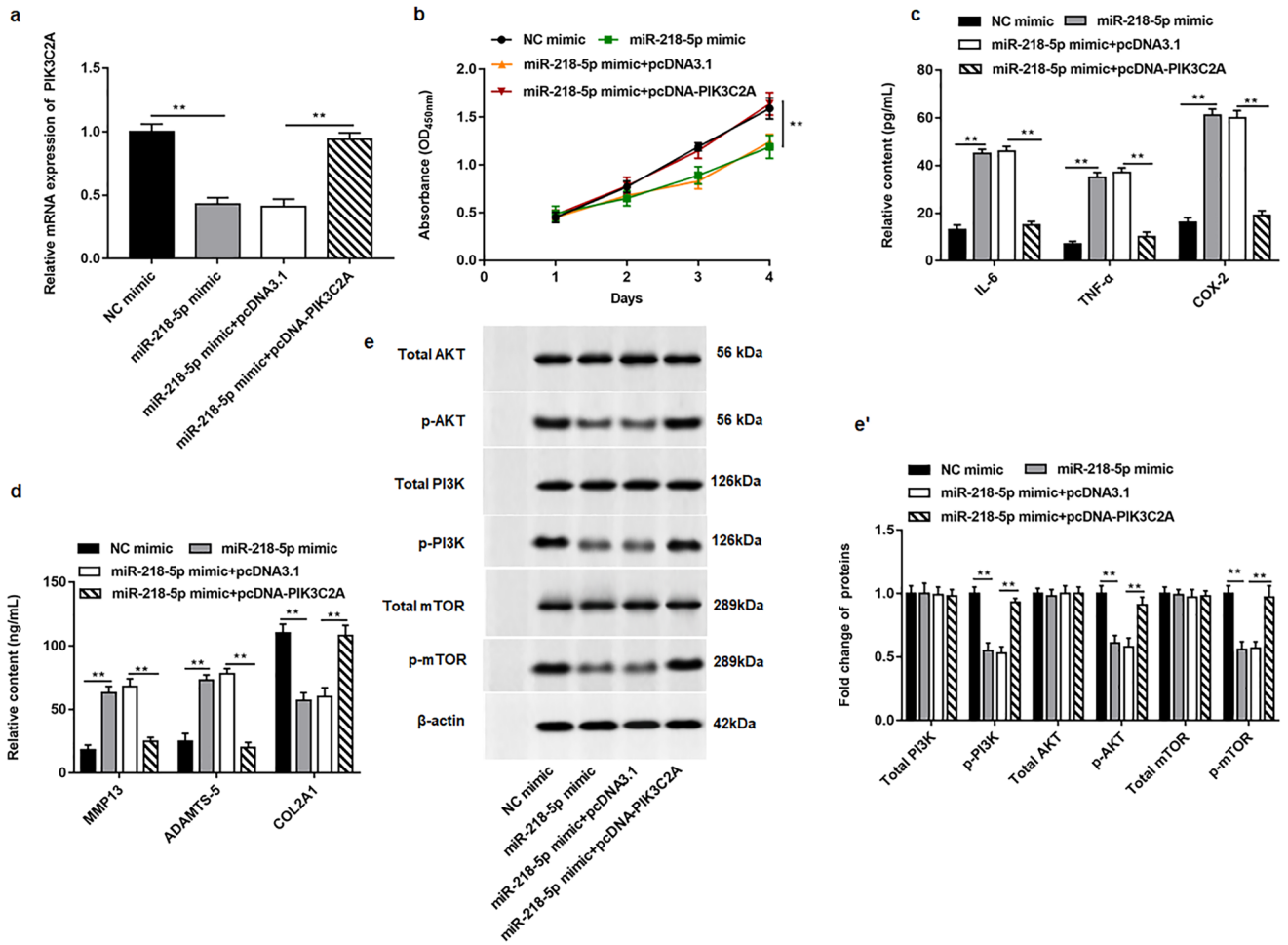

Fig. 6 MiR-218-5p regulated proliferation and apoptosis of osteoarthritic chondrocytes by targeting PIK3C2A. Osteoarthritic chondrocytes were transfected with miR-218-5p mimic alone or together with pcDNA-PIK3C2A, respectively. **a** Relative mRNA expression of PIK3C2A was analyzed by RT-qPCR. **b** Osteoarthritic chondrocyte viability was analyzed by MTT assay. **c** Relative content of IL-6, TNF-α, and COX-2 were analyzed by ELISA kits, respectively. **d** ELISA was used to detect the secretion levels of MMP-13, ADAMTS-5, and COL2A1. **e** and **e′** The protein expression of total PI3K, p-PI3K, total AKT, p-AKT, total mTOR, and p-mTOR in osteoarthritic chondrocytes was analyzed by Western botting. β-actin was used as an invariant internal control for calculating protein-fold changes. $N=3$, $^{**}P<0.01$

mimic reversed the decreased effect of KCNQ1OT1 on MMP-13 and ADAMTS-5 and the increased effect of COL2A1 (Fig. 7d). Besides, Western blotting results showed that the phosphorylation levels of PI3K, Akt, and mTOR were increased when KCNQ1OT1 mimic was overexpress, whereas miR-218-5p mimic significantly reversed the increased effect of KCNQ1OT1 on the phosphorylation levels of PI3K, Akt and mTOR (Fig. 7e, e′).

Discussion

In this study, we found that KCNQ1OT1 expression was decreased in osteoarthritic chondrocytes. Overexpression of KCNQ1OT1 could promote the proliferation of osteoarthritic chondrocytes and reduce their apoptosis. However, the specific mechanism of KCNQ1OT1 in osteoarthritis needs further investigation. Li et al. showed that KCNQ1OT1 expression was upregulated in bladder cancer tissues compared with paracancerous tissues, and they confirmed that KCNQ1OT1 could specifically bind to miR-218-5p and reduce its expression, while overexpression of miR-218-5p inhibited bladder cancer cell proliferation and metastasis and promoted cell apoptosis. Mechanistically, they found that kcnq1ot1 could promote bladder cancer development via miR-218-5p and its target gene HS3ST3B1 (Li et al. 2020). Recently, there is new evidence that KCNQ1OT1 is significantly up-regulated in osteogenic differentiation of bone marrow mesenchymal stem cells (BMSCs), while KCNQ1OT1 gene knockout inhibits osteogenic differentiation

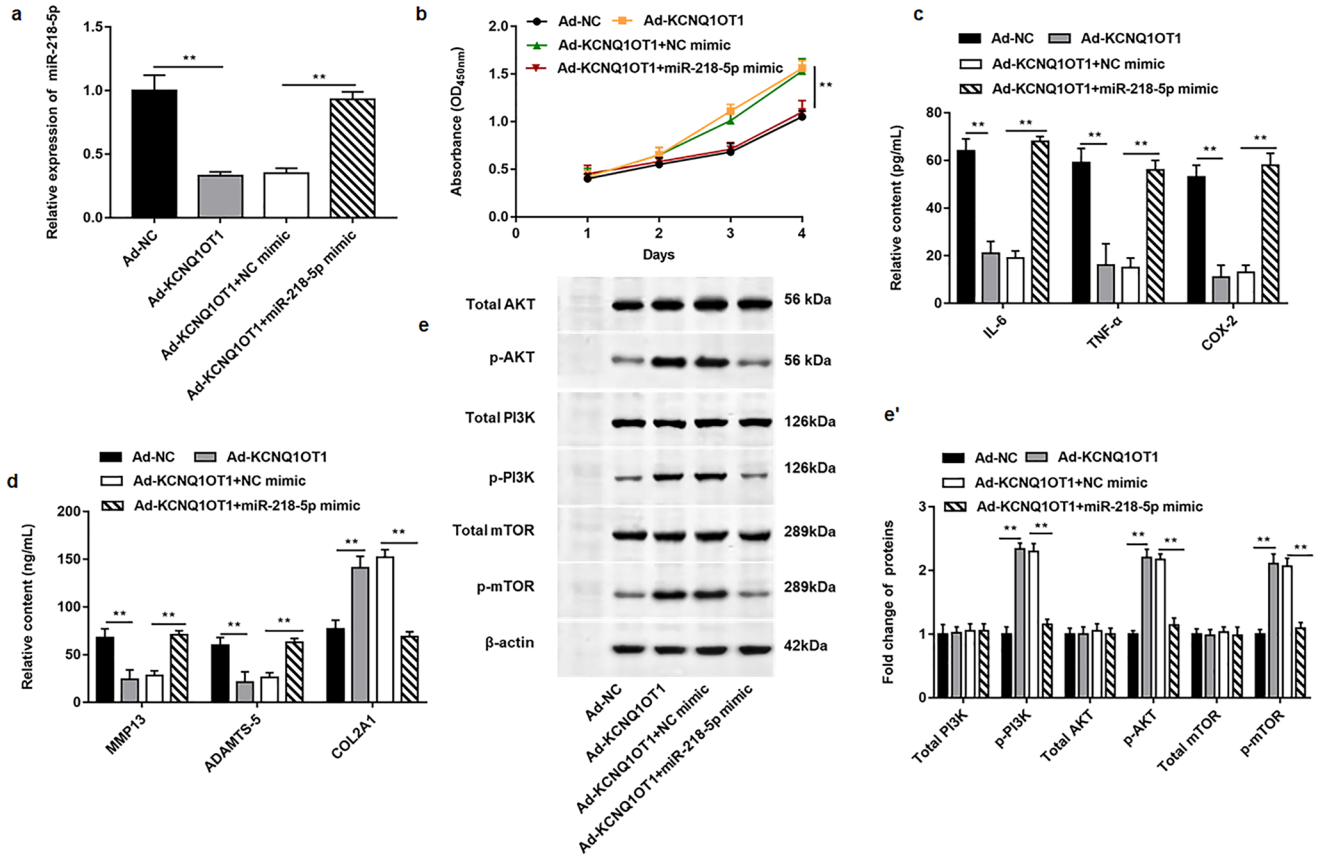

Fig. 7 KCNQ1OT1 attenuated chondrocyte dysfunction by activating the PI3K/Akt/mTOR pathway by targeting miR-218-5p. The Ad-KCNQ1OT1 was transfected into osteoarthritic chondrocytes alone or together miR-218-5p mimic, respectively. **a** Relative expression of KCNQ1OT1 was analyzed by RT-qPCR. **b** MTT assay was used to measure the viability of osteoarthritic chondrocytes. **c** IL-6, TNF-α, and COX-2 content were analyzed by ELISA kits, respectively. **d** The secretion levels of MMP-13, ADAMTS-5, and COL2A1 was detected by ELISA. **e** and **e′** The protein expression of total PI3K, p-PI3K, total AKT, p-AKT, total mTOR, and p-mTOR in osteoarthritic chondrocytes was analyzed by Western botting. β-actin was used as an internal reference. $N=3$, $^{**}P<0.01$

and downregulates the expression of bone formation related genes, suggesting that KCNQ1OT1 has a positive regulatory effect on osteogenic differentiation of BMSCs (Wang et al. 2019). Gao et al. found that KCNQ1OT1 could directly target miR-21a-5p to promote the polarization of primary bone marrow-derived macrophages (BMMs) and improve the osteolysis induced by polymethylmethacrylate particles (Gao et al. 2018). The above studies suggest potential regulatory roles in bone diseases and provide ideas for our research.

We found that miR-218-5p is a potential target of KCNQ1OT1, and the dual luciferase reporter gene assay confirmed the targeting relationship between miR-218-5p and KCNQ1OT1. A study has shown that miR-218-5p mimic can significantly reduce the symptoms of postmenopausal osteoporosis (PMOP) mice, and compared with normal bone marrow mesenchymal stem cells (BMMSCs), the activity of ALP and miR-218-5p expression in osteogenic differentiation induced by BMMSCs in PMOP mice decreased significantly. However, the transfection of miR-218-5p mimic and inhibitor significantly promoted and inhibited the differentiation of PMOP-BMMSCs (Kou et al. 2020). Another study showed that the expression of miR-218-5p was significantly up-regulated in the bone tissue of patients with glucocorticoid induced osteoporosis, while the expression of genes related to osteoblast function and maturation including COL1A2, BMP2, and RUNX2 was down regulated, suggesting that miR-218-5p may become a further research object for the treatment and intervention of the disease (Belaya et al. 2018). Furthermore, Lu et al. found that miR-218-5p was significantly upregulated in moderate and severe osteoarthritic cartilage tissues, and PIK3C2A was identified as a target of miR-218-5p in SW1353 and C28/I2 cells. They found that miR-218-5p mimics significantly inhibited chondrocyte collagen II and aggrecan secretion and promoted MMP-13 and ADAMTS-5 secretion. In the mechanism study, they found that downregulation of miR-218-5p promoted the expression of PIK3C2A and its downstream

target proteins including Akt and mTOR. In vivo studies revealed that osteoarthritic mice exposed to miR-218-5p inhibitor exhibited less articular chondrocyte loss than control mice(Lu et al. 2017). Taken together, the study by Lu et al. revealed a significant finding of miR-218-5p in osteoarthritis and was further validated in the present study.

Furthermore, we found that miR-218-5p was upregulated in osteoarthritic chondrocytes and directly targets 3′UTR of PIK3C2A. PIK3C2A is a class II member of the phosphoinositide 3-kinase (PI3K) family that catalyzes the phosphorylation of phosphatidylinositol (PI) into PI(3)P and the phosphorylation of PI(4)P into PI(3,4)P2. It has been reported that PIK3C2A was upregulated in osteosarcoma compared with para carcinoma bone tissues, and high levels of PIK3C2A were closely associated with osteosarcoma metastasis and poor overall survival (Chao et al. 2018). Furthermore, researchers found that the deletion mutation of PIK3C2A in children of three independent close relatives could lead to short stature and multiple skeletal abnormalities, suggesting the key role of class II PI3K in growth and bone formation (Tiosano et al. 2019).

The PI3K/AKT/mTOR signaling pathway has been known as one of the most prominent signal cascades to regulate cell survival, differentiation and development (Song et al. 2019; Sun et al. 2020). Of note, several previous studies have demonstrated that this signaling pathway plays a critical role in osteoarthritis pathogenesis. Wang et al. found that miR-138-5p regulated chondrocyte proliferation and alleviates intervertebral disc degeneration through the PTEN/PI3K/AKT signaling pathway (Wang et al. 2016). He et al. showed that inhibition of miR-20 expression can promote the proliferation of articular chondrocytes via the PI3K/AKT/mTOR axis (He and Cheng 2018). Xu et al. reported that resveratrol inhibited the development of obesity-related osteoarthritis via the TLR4 and PI3K/AKT signaling pathways (Xu et al. 2019). In this study, we found that KCNQ1OT1 was significantly down regulated in osteoarthritic chondrocytes. Overexpression of KCNQ1OT1 promoted the proliferation of osteoarthritic chondrocytes and inhibited apoptosis and the secretion of inflammatory factors. Furthermore, our results clearly showed that KCNQ1OT1 can directly adsorb miR-218-5p to regulate PIK3C2A expression. The overexpression of KCNQ1OT1 and PIK3C2A in osteoarthritic chondrocytes resulted in a significant increase in the phosphorylation levels of PI3K, Akt, and mTOR, while miR-218-5p mimic significantly reversed the regulatory effect of KCNQ1OT1 and PIK3C2A.

In this study, we demonstrate that KCNQ1OT1 upregulates PIK3C2A and activates the PI3K/AKT/mTOR pathway to reduce chondrocyte dysfunction by targeting miR-218-5p. Our findings may provide new ideas for the treatment of osteoarthritis.

Declarations

Ethical approval The present study and the associated experimental protocols (human experiments) were performed in compliance with ethical guidelines and approved by the Institute Research Medical Ethics Committee of the First Hospital of Jilin University, Changchun, Jilin, China. (approval no. FH2016-184). All osteoarthritis tissues and non-osteoarthritis tissues were also used in accordance with the Helsinki declaration.

Informed consent Fully informed consent was obtained from every participant prior to enrollment.

Conflict of interest The authors declare that they have no conflict of interest.

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